12 Oct 2000

Set	Items	Description
S1	85070	(PCR OR POLYMERSE()CHAIN)/TI
S2	0	S1 AND AU=DAU
S3	0	S1 AND AU=DAY
S4	1153	S1 AND DAY/AU
S5	303	RD (unique items)
S6	1	S5 AND LIGASE AND RESTRICTION
S7	65	S1 AND LIGASE AND RESTRICTION
S8	29	RD (unique items)
S9	1	S8 AND DAY
S10	0	S9 NOT S6
S11	128	S1 AND LCR
S12	61	RD (unique items)
S13	0	S12PY=1999
S14	10856	S1 AND PY=1999
S15	1312	S14 AND RESTRICTION
S16	36	S15 AND LIGAT?
S17	10	RD (unique items)
S18	1	S17 AND DAY

Multiplex %PCR%/LDR for detection of K-ras mutations in primary colon tumor.

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ABSTRACT: Point mutations in codons 12, 13, and 61 of the K-ras, gene occur early in the development of colorectal cancer and are preserved throughout the course of tumor progression. These mutations can serve as biomarkers for shed or circulating tumor cells and may be useful for diagnosis of early, curable tumors and for staging of advanced cancers. We have developed a multiplex %polymerase% %chain% reaction/ligase detection reaction (%PCR%/LDR) method which identifies all 19 possible single-base mutations in K-ras codons 12, 13, and 61, with a sensitivity of 1 in 500 wild-type sequences. In a blinded study, 144 paraffin-embedded archival colon carcinomas were microdissected and K-ras mutations determined by both dideoxy-sequencing and multiplex %PCR%/LDR. Results were concordant for 134 samples. The ten discordant samples were re-evaluated using higher sensitivity uniplex %PCR%/LDR, and the original multiplex %PCR%/LDR result was confirmed in nine of these ten cases. Multiplex %PCR%/LDR was able to identify mutations in solid tumors or paraffin-embedded tissues containing a majority of wild-type stromal cells, with or without microdissection. The technique is well suited for large scale studies and for analysis of clinical samples containing a minority population of mutated cells.

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Universal DNA microarray method for multiplex detection of low abundance point mutations.
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ABSTRACT: Cancers arise from the accumulation of multiple mutations in genes regulating cellular growth and differentiation. Identification of such mutations in numerous genes represents a significant challenge in genetic analysis, particularly when the majority of DNA in a tumor sample is from wild-type stroma. To overcome these difficulties, we have developed a new type of DNA microchip that combines %polymerase% %chain% reaction/ligase detection reaction (%PCR%/LDR) with "zip-code" hybridization. Suitably designed allele-specific LDR primers become covalently ligated to adjacent fluorescently labeled primers if and only if a mutation is present. The allele-specific LDR primers contain on their 5'-ends "zip-code complements" that are used to direct LDR products to specific zip-code addresses attached covalently to a three-dimensional gel-matrix array. Since zip-codes have no homology to either the target sequence or to other sequences in the genome, false signals due to mismatch hybridizations are not detected. The zip-code sequences remain constant and their complements can be appended to any set of LDR primers, making our zip-code arrays universal. Using the K-ras gene as a model system, multiplex %PCR%/LDR followed by hybridization to prototype 3 X 3 zip-code arrays correctly identified all mutations in tumor and cell line DNA. Mutations present at less than one per cent of the wild-type DNA level could be distinguished. Universal arrays may be used to rapidly

Nucleotide analogs facilitate base conversion with 3' mismatch primers.

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We compared the efficiency of %PCR% amplification using primers containing either a nucleotide analog or a mismatch at the 3' base. To determine the distribution of bases inserted opposite eight different analogs, 3' analog primers were used to amplify four different templates. The products from the reactions with the highest amplification efficiency were sequenced.Analogs allowing efficient amplification followed by insertion of a new base at that position are herein termed 'convertides'. The three convertides with the highest amplification efficiency were used to convert sequences containing C, T, G and A bases into products containing the respective three remaining bases. Nine templates were used to generate conversion products, as well as non-conversion control products with no base change. We compared the ability of natural bases to convert specific sites with and without a preconversion step using nucleotide analog primers. Conversion products were identified by a ligation detection reaction using primers specific for the converted sequence. We found that conversions resulting in transitions were easier to accomplish than transversions and that sequence context influences conversion. Specifically, primer slippage appears to be an important mechanism for producing artifacts via polymerase extension of a 3' base or analog transiently base paired to neighboring bases of the template. Nucleotide analogs could often reduce conversion artifacts and increase the yield of the expected product. While new analogs are needed to reliably achieve transversions, the current set have proven effective for creating transition conversi